REMARKS

Claims 20 and 24-26 were pending in the subject application. The subject matter of claim 26 is allowable. By this amendment, applicants have amended claims 24 and 25, and added new claims 27-38. Applicants maintain that the amendments to the claims do not raise an issue of new matter. Support for the amendment to claim 24 can be found in the specification at least on page 3, lines 24-29, and page 7, line 18 through page 8, line 18. Support for the amendment to claim 25 can be found in the specification at least on page 8, lines 19-35. Support for new claims 27-29 and 33-35 can be found in the specification at least on page 5, lines 31-34. Support for new claims 30-32 and 36-38 can be found in the specification at least on page 6, lines 22-36. Accordingly, applicants respectfully request that the Amendment be entered.

Rejections under 35 U.S.C. §112, Second Paragraph

Claims 24 and 25 are rejected as indefinite under 35 U.S.C. §112, second paragraph.

Applicants have hereinabove rewritten Claim 24 to recite steps for determining erythrocyte adenylate kinase activity and have amended Claim 25 to recite that the antibody binds to adenylate kinase. Accordingly, reconsideration and withdrawal of this ground of rejection are respectfully requested.

Rejections under 35 U.S.C. §103(a)

a) Claims 20 and 24

Claims 20 and 24 stand rejected under 35 U.S.C. §103(a) as unpatentable over Olsson et al., 1983, J. Appl. Biochem. 5:437-445.

Applicants respectfully traverse this rejection, and maintain that the claimed invention is patentable over Olsson for reasons which follow.

Olsson measured total adenylate kinase activity in the plasma of stored units of red blood cells and found that the activity correlated with hemoglobin release from the red cells in the storage bag. In contrast, the present invention is directed to a method for diagnosing erythrocyte hemolysis in a subject. In the present invention, hemolysis of red blood cells takes place *in vivo*. The natural clearance of erythrocyte adenylate kinase from the blood that occurs *in vivo* does not take place in a storage bag. In addition, adenylate kinase also can enter the blood from sources other than erythrocytes, such as the liver, intestine and muscles (see, e.g., first full paragraph on page 2 of the specification). These extra sources of adenylate kinase do not exist in a blood storage bag. Based upon these distinctions alone, the claimed invention is patentable over Olsson.

The premise of the obviousness rejection is based on the presumption that levels of blood components present in the serum of stored blood will behave similarly in the serum of patients with hemolysis. This is not necessarily so. Although Olsson indicates that hemoglobin levels correlate with hemolysis of stored blood, such a correlation does not necessarily exist in the serum of patients with hemolysis. When hemoglobin is discharged directly into the circulation from destroyed red blood cells, the released hemoglobin is removed by several mechanisms (see Deiss, A. Destruction of Erythrocytes, In: Wintrobe's Clinical Hematology 10th Edition, Lee GR, Foerster J, Lukens J, Paraskevas F, Greer JP, Rodgers GM Eds. Williams and Wilkins, Baltimore 1998, pages 276-279, Figure 12.4, **Exhibit 1)**. At low rates of release of hemoglobin into plasma, all of the hemoglobin is found to be attached to the plasma protein haptoglobin (see *id.* at

p. 276, right column). The haptoglobin-hemoglobin complex is removed by hepatic parenchymal cells (see *id.* at p. 276, Fig. 12.4 legend). When the haptoglobin becomes saturated, free unbound hemoglobin circulates briefly in plasma (*id.* at p. 278, right column, last paragraph). The free unbound hemoglobin is removed by three different mechanisms: 1) removal by hepatic parenchymal cells, 2) excretion by the kidney, and 3) oxidization to methemoglobin (*id.* at p. 276, Fig. 12.4 legend; p. 278, right column; p. 279, left column). Thus, with low levels of hemolysis, the plasma hemoglobin level does not correlate with hemolysis. In addition, in a classical clinical study more than 50 years ago, it was demonstrated that plasma hemoglobin levels are normal in patients with most hereditary hemolytic anemias (Crosby WH, Dameshek W. The significance of hemoglobinemia and associated hemosiderinuria, with particular reference to various types of hemolytic anemia. J Lab Clin Med 1951; 38:829-41, see Table I on page 832, **Exhibit 2**). These authors commented that "[t]aken alone, the plasma hemoglobin was of little significance in assessing the severity of the hemolytic disease." See *id.* at pp. 836-837. Therefore, notwithstanding the teachings of Olsson that elevated hemoglobin corresponds to hemolysis of stored blood, hemoglobin is not an effective marker for low levels of hemolysis *in vivo*.

There are a number of disadvantages associated with currently used tests for *in vivo* hemolysis (reviewed in Introduction of Burns ER, Kale A, Murthy VV, Diagnosis of the hemolytic state using serum levels of erythrocyte adenylate kinase. Am. J. Hematol. 84: 180-183, 2000, **Exhibit 3**, and in Discussion of Thomas G and Murthy VV, Erythrocyte adenylate kinase isoenzyme as a marker for hemolysis, J. Clin. Lab. Anal. 11: 351-356, 1997, **Exhibit 4**). For example, serum lactate dehydrogenase levels, which are elevated in hemolysis, may also be elevated in hepatic, cardiac, pulmonary, and

placental diseases, thus reducing the test's specificity for detecting hemolysis (Thomas and Murthy at p. 356). In addition, lactate dehydrogenase levels are inconsistently variable in conditions of extravascular hemolysis (Burns et al. at p. 180). Similarly, the bilirubin test is also deficient is specificity since serum bilirubin levels may be elevated in intra- and extra-hepatic biliary disease and in severe hepatocellular dysfunction (Thomas and Murthy at p. 356). The bilirubin test is also insensitive, because bilirubin levels are normal in almost half of patients with immune hemolytic anemia (Burns et al. at p. 180). The haptoglobin test is also nonspecific because haptoglobin can rise to normal levels in the presence of hemolysis when inflammatory conditions are present (*ibid.*). The reticulocyte count is often elevated in hemolytic conditions, but this indicator is often blunted in the face of chronic disease, renal failure, chemotherapy, radiotherapy, or bone marrow dysfunction, making it too insensitive as an indicator of hemolysis. The absence of reticulocytosis cannot reliably exclude hemolysis (*id.* at p. 180 and 182). Finally, the ^{51}chromium red cell survival test is too cumbersome for routine rapid use (*id.* at p. 180).

The claimed method offers advantages over currently used tests for *in vivo* hemolysis that attest to the nonobviousness of the subject invention. MPEP §2141. The superior analytic sensitivity of the present method allows the detection of even marginal hemolysis in hemolytic anemia patients, where such anemia is not apparent using a plasma hemoglobin assay (see Thomas and Murthy, Table 2 on page 354, and page 356, right column). The sensitivity, specificity and likelihood ratios of the present erythrocyte adenylate kinase (EAK) assay for the diagnosis of hemolysis are compared to those for the bilirubin, lactate dehydrogenase, and reticulocyte count assays in Table I on page 182 of Burns et al. Using the upper limit of the 95% confidence interval for each marker, the EAK assay had the best combined sensitivity (96%) and specificity (97%) (*id.* at p.

181, last paragraph). Receiver operating characteristics analysis of the four different assays indicated that EAK had the best diagnostic utility (*id.* at p. 181, last paragraph and Fig. 2 on p. 182). Table II on page 182 of Burns et al. demonstrates that the EAK assay showed no overlap between subjects with hemolysis and those with either acute myocardial infarction (M.I.) or those with hepatic dysfunction ("Medically Ill"). In contrast, the bilirubin and lactate dehydrogenase assays yield a range of values that overlaps among the three patient groups, thus limiting the clinical utility of these assays (*id.* at p. 181, last paragraph).

In view of the remarks made hereinabove, applicants submit that the claimed invention is patentable over Olsson. Accordingly, reconsideration and withdrawal of this ground of rejection are respectfully requested.

b) Claim 25

Claim 25 stands rejected under 35 U.S.C. §103(a) as unpatentable over Olsson et al., 1983, J. Appl. Biochem. 5:437-445, in view of Matsuura et al., 1989, J. Appl. Biochem. 264:10148-55. Applicants respectfully traverse this rejection. Applicants maintain that Claim 20 is patentable over Olsson for the reasons set forth herein above. Claim 25 depends from, and further limits, Claim 20. Accordingly, applicants maintain that Claim 25 is patentable over the cited references, and respectfully request that the Examiner reconsider and withdraw this ground of rejection.

Supplemental Information Disclosure Statement

In accordance with the duty of disclosure under 37 C.F.R. §1.56, applicants would like to direct the Examiner's attention to the references listed on the attached form PTO/SB/08B and attached hereto (**Exhibit 5**). Applicants are submitting this IDS pursuant to 37 C.F.R. §1.97(c)(2) before the mailing of any of a Final Office Action, a Notice of Allowance, or an action that otherwise closes prosecution in the application. A check is enclosed to cover the $180.00 fee for submitting an IDS pursuant to 37 C.F.R. §1.97(c)(2).

CONCLUSIONS

In view of the amendments and remarks made herein above, reconsideration and withdrawal of the rejections in the May 18, 2004 Office Action and passage of the pending claims to allowance are respectfully requested. If there are any minor matters that would prevent allowance of the subject application, the Patent Office is requested to telephone the attorneys listed below.

A check for $395.00 is enclosed to cover the $215.00 fee for a two month extension of time for a small entity and the $180.00 fee for filing an Information Disclosure Statement. No other fee is deemed necessary in connection with the submission of this response. However, if any other fee is required to maintain the pendency of the subject application, the Patent Office is authorized to withdraw the amount of any such fee from Deposit Account No. 01-1785. Overpayments may be credited to Deposit Account No. 01-1785.

Respectfully submitted,

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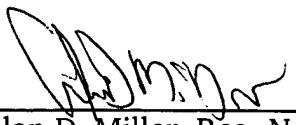
October 14, 2004

By: 
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